Remarks/Arguments

Reconsideration of the above-identified application in view of the present amendment is respectfully requested.

By the present amended claim 6 has been canceled. Claim 5 has been amended to recite that the human mesenchymal stem cells are a homogenous population of mesenchymal stem cells that have isolated, purified, and then culturally expanded from human mesoderm tissue. Support for this limitation can be found on page 9 lines 25-30 and page 10, lines 1-10.

Additionally, new claim 7 has been added that recites the homogenous population of mesenchymal stem cells uniformly express SH2, SH3, and SH4 and lack surface markers for T and B lymphocytes, macrophages, and endothelial cells. Support for these limitations can be found on page 5, lines 14-17, and page 10, lines 1-5.

Below is a discussion of the Specification objections, the 35 U.S.C. §112, first paragraph rejection of claim 6, and the 35 U.S.C. §102(b) rejections of claims 2-6.

1. Specification Objection

The specification is objected to as failing to provide proper antecedent basis for the claimed subject matter. Correction of the phrase "human mesenchymal stem cells that have been isolated, purified and culturally expanded from bone marrow specimen by adding the bone marrow specimen to a medium which contains factors which stimulate mesenchymal cell growth without differentiation" recited in claim 6. As stated above, claim 6 has been canceled. Therefore, applicants respectfully submit that the objection to the specification is moot.

2. 35 USC §112, first paragraph, rejection of claim 6

Claim 6 is rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The Office Action states that the claim contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. While the Applicants respectfully disagree, in the interest of furthering prosecution claim 6 has been canceled. Therefore, Applicants respectfully submit that the rejection to claim 6 is now moot.

3. 35 USC §102 rejection of claims 3-6

Claims 3-6 are rejected under 35 U.S.C. §102(b) as being anticipated by Nolta *et al.* (Blood 86:101-110, 1995, Cited previously) as evidenced by Prockop, D.J. (Science 276:71-74, 1997; Cited previously) and/or (Mesenchymal stem cell-Wikipedia, the free encyclopedia, page 1-5, 2009) for the same reasons already set forth in the Office Action mailed on 6/25/2009 (pages 5-6).

The Office Action argues that Nolta *et al.* disclose a transduction method for human CD34 cells isolated form bone marrow and peripheral blood with retroviral vectors containing either the bacterial neo gene, or normal human glucocerebrosidase in the presence of a stroma generated by 4^{th} passaged human allogeneic bone marrow stromal cells prior to the plating of CD34 cells. The utilized bone marrow stromal cell population derived from bone marrow spicules in devoid of most hematopoietic cells, and it contains isolated mesenchymal cells or isolated multipotential bone marrow stromal cells as evidenced by the teachings of Prockop,

including the disclosure that the adherent cells used as feeder layers for hematopeoietic stem cells have many of the characteristics of MSCs isolated by their adherence to plastic in the absence of non-adherent cells. The Office Action further argues that the terms "Mesenchymal stem cell" and "Marrow stromal cell" have been used interchangeably in the art as evidenced by Wikipedia, the free encyclopedia. Thereby concluding that the bone marrow stromal cells that were passage 4 times for transduction as taught by Nolta *et al.* are mesenchymal stem cells that have been isolated, purified, and culturally expanded from human mesoderm tissue.

Applicants respectfully submit that claims 3-5 are not anticipated by Nolta *et al.* as evidenced by Prockop and/or Wikipedia, the free encyclopedia because Nolta *et al.* do not teach a homogenous population of mesenchymal stem cells that have been isolated, purified, and then culturally expanded, as claimed in the present application. Specifically, Applicants note that Nolta et al. do not teach a homogenous population of human mesenchymal cells that have been isolated, purified, and then culturally expanded from human mesoderm tissue and the claimed isolated, purified and then culturally expanded homogenous population of mesenchymal stem cells are not the same population of cells as taught by Nolta *et al.*

In order for a claim to be anticipated each and every element of the claimed invention, operating in the same fashion to form the identical function as the claimed product must be literally present in a single prior art reference, arranged as in the claim. *Scripps Clinic & Research Found. v. Genentech, Inc.* 927 F.2d 1565, 1576 (Fed. Cir. 1991); Carella v. Starlight Archery & Pro Line Co., 804 F.2d at 138. "There

must be no difference between the claimed invention and the referenced disclosure, as viewed by a person of ordinary skill in the field of the invention." *Scripps Clinic & Research Found. v. Genentech, Inc., 927 F.2d at 1576; see also E.I. Du Pont de Nemours & Co. v. Polaroid Graphics Imaging. Inc.,* 706 F. Supp. 1135, 1142 (D. Del.1989), (aff'd, 887 F.2d 1095 (Fed. Cir. 1989) ("all of the same elements [must be] found in exactly the same situation and united in the same way ... in a single prior art reference") (quoting *Perkin Elmer Corp. v. Computervision, Corp.,* 732 *F.2d* 888, 894 *(Fed. Cir. 1984)).* It is not sufficient that each element be found somewhere in the reference, the elements must be "arranged as in the claim." *Lindemann Maschinenfabrik GmbH v. American Hoist & Derrick Co.,* 730 F.2d 1452, 1458 (Fed. Cir. 1984). Thus, any degree of physical difference between the patented product and the prior art, *no matter how slight,* defeats the claim of anticipation. *E.I. Du Pont de Nemours & Co. v. Polaroid Graphics Imaging, Inc.,* 706 F. Supp. at 1142.

Nolta *et al*. teaches that,

> spicules from unseparated bone marrow were collected by gravity sedimentation and plated in stromal medium… [and then] subconfluent layers of primary stromal cells were split by trypsinization. Stroma was not used as a supporting layer for transduction until passage no. 4. At [that] point, most hematopoietic cells had been eradicated, except for mature macrophages, which comprised less than 1% of the culture, as shown by fluorescence-activated cell sorter (FACS) analysis and immunohistochemical staining for the panleukocyte antigen CD45 using the monoclonal antibody HLE-1 as described. Stromal cells were irradiated (20Gy) and plated at 5 x 10^5 cells per T25 vent-cap flask in IMDM/20% FCS the day before use."

(p102, col 1, 2nd full para.). The result of the isolation technique taught by Nolta *et al*. is a heterogeneous, poorly defined population of marrow derived cells. There is

nothing in Nolta et al. that states that this population is a homogenous population of mesenchymal stem cells let alone an isolated and purified population that has been culturally expanded. The mere fact Nolta et al. teach the stromal cells have been passaged only suggests that that an isolated heterogenous population has been isolated. There is nothing in Nolta et al. that teaches this isolated population is homogenous and/or is subsequently culturally expanded.

In contrast claim 5 recites a homogenous population of mesenchymal stem cells that have been isolated, purified, and then culturally expanded. The specification of the application which is used construe this language teaches at p. 5, ll. 1-12 that:

> [m]esenchymal stem cells (MSCs) can be derived from marrow, periosteum, dermis and other tissues of mesodermal origin. The mesenchymal stem cells can be isolated and prepared according to methods known in the art, for example, a process for isolating, purifying, and expanding the marrow-derived mesenchymal stem cells in culture, i.e. in vitro, is described in U.S. Patent Nos. 5,197,985 and 5,226,914 and PCT Publication No. WO 92/22584 (1992) as well as numerous literature references by Caplan and Haynesworth. The stem cells may be isolated from other cells by density gradient fractionation, such as by Percoll gradient fractionation. The human mesenchymal stem cells also can include a cell surface epitope specifically bound by antibodies from hybridoma cell line SH2, deposited with the ATCC under accession number HB10743l antibodies form hybridoma cell line SH3, deposited with ATCC under accession number HB10744; or antibodies from hybridoma cell line SH4, deposited with the ATCC under accession number HB10745 (Emphasis added).

Applicants note that the mesenchymal stem cells taught in the present application are a specific cell type, possessing defined characteristics recognized by those skilled in the art, as evidenced by Majumdar *et al.* (J. Cell. Phys. 178:57-66,1998; Enclosed). Majumdar *et al.* discuss the differences between isolated, purified mesenchymal stem cells (MSCs) and marrow-derived stromal cells (MDSCs)

(see Abstract). Majumdar *et al.* teach that MSCs are morphologically distinct from MDSC cultures, and flow cytometric analyses show that MSCs are a homogeneous cell population (*Id.*; Figs. 1 & 2). In addition, MSCs were found to have greater steady state levels of cytokine expression than MDSCs (*See* Abstract and Fig. 3). Majumdar *et al.* disclose a method for isolating, purifying and culturally expanding MSCs that utilizes Percol density centrifugation as a purification step (p58, first full para.). The use of Percol purification is discussed in the present specification at p. 5, ll. 6-7 and p. 9, ll. 29-30.

Applicants further note that the present application differentiates between MSCs and Dexter stroma. Dexter stroma as discussed in the present specification is the same as the MDSCs discussed by Majumdar *et al.* (See Majumdar *et al.* p58, para. spanning col. 1-2 and Experimental Example p10, ll. 13-23.). Thus, one skilled in the art would recognize that MSCs are a distinct cell population from the heterogeneous stromal cells discussed by Nolta *et al.*

In addition, while there are several art-recognized methods for arriving at a homogenous population of mesenchymal stem cells that have been isolated, purified, and culturally expanded, the present application provides examples of specific methods as noted above.

Nolta *et al.* teach the isolation of adherent marrow cells from non-adherent cells. However, Nolta *et al.* do not characterize or demonstrate that the passaged adherent cells are a homogeneous population of mesenchymal stem cells or that there is even a high proportion of mesenchymal stem cells compared to other cell types. In fact, Nolta *et al.* do not discuss mesenchymal stem cells at all. Moreover,

there is nothing in Nolta et al. that teaches the passaged stromal cells of Nolta et al. would inherently be a homogenous population of mesenchymal stem cells.

The Office Action infers that mesenchymal stem cells are isolated, purified, and expanded by referencing Prockop as support for the presence of mesenchymal stem cells in the adherent cell population. However, Prockop does not support the argument that mesenchymal stem cells have been isolated, purified, and expanded. Contrary to the position taken by the Office Action, Prockop teaches that there are advantages to utilizing isolation and purification methods as opposed to the "crude procedure of Freidenstein," *i.e.,* the isolation of marrow stromal cells via adherence to plastic alone that is also used by Nolta *et al.* (see p72, col. 1, para. 3 and col. 2, para. 2). These advantages include that "the isolated cells are either clonal or nearly clonal, they express small amounts of bone cell markers such as alkaline phosphatase, and they can be induced in culture to express large amounts of the same markers…" (p72, col. 2, para 2).

In addition, the Office Action argues that the terms "Mesenchymal stem cell" and "Marrow stromal cell" have been used interchangeably in the art as evidenced by Wikipedia, the free encyclopedia. However, Applicants respectfully submit that the statements found in the cited Wikipedia page are not factually accurate and a person of skill in the relevant art would recognize that a homogenous population of mesenchymal stem cells that have been <u>isolated, purified, and then culturally expanded</u> are distinct from the marrow stromal cells taught by Nolta *et al.*

As discussed above, Majumdar *et al.* discuss the differences between isolated, purified mesenchymal stem cells (MSCs) and marrow-derived stromal cells

(MDSCs) (see Abstract). Majumdar *et al.* teach that MSCs are morphologically distinct from MDSC cultures, and flow cytometric analyses show that MSCs are a homogeneous cell population (*Id.*; Figs. 1 & 2). In addition, MSCs were found to have greater steady state levels of cytokine expression than MDSCs (*See* Abstract and Fig. 3). Majumdar *et al.* disclose a method for isolating, purifying and culturally expanding MSCs that utilizes Percol density centrifugation as a purification step (p58, first full para.). The use of Percol purification is discussed in the present specification at p. 5, ll. 6-7 and p. 9, ll. 29-30.

Applicants note that the present application differentiates between MSCs and Dexter stroma. Applicants further note that Dexter stroma as discussed in the present specification is the same as the MDSCs discussed by Majumdar *et al.* (See Majumdar *et al.* p58, para. spanning col. 1-2 and Experimental Example p10, ll. 13-23.). Thus, one skilled in the art would recognize that MSCs are a distinct cell population from the heterogeneous stromal cells discussed by Nolta *et al.*

The Examiner notes that the instant specification states "These results demonstrate that hMSCs are able to support ex vivo gene transfer into CD34 human hematopoietic progenitor cells that exhibit transduction efficiencies, cell expansion and drug resistance comparable to the levels produced in Dexter Stroma and FN enhanced transduction", and that "Dexter Stroma was derived from adhered bone marrow mononuclear cells that were passaged once". The statements cited by the Examiner were not intended to, nor do they illustrate that the cell populations of Dexter Stroma and the MSCs isolated from human mesoderm tissue are substantially identical cell populations. This statement was merely included in the

present Application to illustrate the effectiveness of the present invention in relation to other methods of ex vivo gene transfer into CD34 human hematopoietic progenitor cells known at the time of the present invention.

The Examiner argues in response to Applicant's previous argument put forth that the stromal cell population is devoid of most hematopoietic cells and contains MSCs as evidenced by Prockop and that this bone marrow derived stromal cell population have many of the characteristics of MSCs.

However, the heterogeneous bone marrow stromal cell population is not a homogenous population of mesenchymal stem cells isolated, purified and then culturally expanded from human mesoderm tissue even if it shares many characteristics with MSCs. Once isolated, purified and culturally expanded, the mesenchymal stem cells of the present Application can be distinguished from the more complex cellular environment present in adherent cells of long-term bone marrow stromal culture as evidenced by Majumdar *et al.* and discussed above.

Therefore, Applicants respectfully submit that the present invention is patentable over Nolta *et al.* as evidenced by Prockop, D.J. and/or Wikipedia because Nolta *et al.* fail to disclose or teach homogenous population of mesenchymal stem cells that have been isolated, purified, and then culturally expanded. Accordingly, Applicants respectfully request that the 35 U.S.C. §102(b) rejection of claim 5 be withdrawn. Claims 3 and 4 depend either directly or indirectly from claim 5, and therefore should be allowed because of the aforementioned deficiencies in the rejection with respect to claim 5 and because of the specific limitation recited in claims 3 and 4.

In addition, claim 6 has been canceled, thereby rendering the §102(b) rejection of claim 6 moot.

4. 35 USC §102 rejection of claims 2 and 4-6

Claims 2 and 4-6 are rejected under 35 U.S.C. §102(b) as being anticipated by Wells *et al.* (Gene Therapy 2:2512-520, 1995) as evidenced by Prockop, D.J. (Science 276:71-74, Cited previously) and/or (Mesenchymal stem cell-Wikipedia, the free encyclopedia, page 1-5, 2009) for the same reasons already set forth in the Office Action mailed on 6/25/2009 (pages 9-11).

The Office Action argues that Wells *et al.* discloses a transduction method for human bone marrow CD34 progenitor cells from a Gaucher patient with retroviral vectors containing a normal human glucocerebrosidase cDNA, in the presence of an autologous bone marrow stromal support containing adherent stromal cells depleted of hematopoietic cells and macrophages that were obtained between passages 3 and 5. The Office Action further argues that the utilized bone marrow stromal support contains isolated mesenchymal stem cells or isolated multipotential bone marrow stromal cells as evidenced by the teachings of Prockop, including the disclosure that the adherent cells used as feeder layers for hematopoietic stem cells have many of the characteristics of MSCs isolated by their adherence to plastic in the absence of non-adherent cells. Furthermore, the terms "Mesenchymal stem cell" and "Marrow stromal cell" have been used interchangeably in the art as evidenced by Wikipedia, the free encyclopedia. Thereby concluding that the bone marrow stromal cells that were obtained between passages 3 and 5 for transduction as

taught by Wells *et al.* are mesenchymal stem cells that have been isolated, purified and culturally expanded form human mesoderm tissue.

Applicants respectfully submit that claim 5 is not anticipated by Wells *et al.* as evidenced by Prockop and/or Wikipedia, the free encyclopedia because Wells *et al.* do not teach a homogenous population of mesenchymal stem cells that have been isolated, purified, and then culturally expanded, as claimed in the present application. Specifically, Applicants note that the claimed homogenous population of mesenchymal stem cells are not the same population of cells taught by Wells *et al.*

Wells *et al.* teach,

> To generate autolgous stromal monolayers, cryopreserved marrow from the patient with Gaucher disease was plated at a concentration of 5 x 10^5 cells/ml in tow types of media. The first medium was IMDM with 15% FCS, 15% HS, 10^{-4} M 2-mercaptoethanol, 10^{-6} M hydrocortisone, 50 U/ml penicillin G, 50 µg/ml streptomycin sulfate, and 2 mM L-glutamine. The second medium was IMDM with 10% FCS, 50 U/ml penicillin G, 50 µg/ml streptomycin sulfate, and 2 mM L-glutamine. All of the non-adherent cells were removed 24h after plating. Subconfluent layers of primary stromal cells were split by trypsinization. Stroma was used as a supporting layer for transduction between passages 3 and 5, after hematopoietic cells and macrophages had been depleted. Stromal cells were irradiated (20Gy) and plated at 5 x 10^5 cells per T-25 vent-cap flask in transduction medium the day before use.

(p518, col. 2, 2nd para). The result of the isolation technique taught by Wells *et al.* is a heterogeneous, poorly defined population of marrow derived cells. There is nothing in Wells et al. that states that this population is a homogenous population of mesenchymal stem cells let alone an isolated and purified population that has been culturally expanded. The mere fact Wells et al. teach the stromal cells have been passaged only suggests that that an isolated heterogenous population has been

isolated. There is nothing in Wells et al. that teach this isolated population is homogenous and/or is subsequently culturally expanded.

In contrast claim 5 recites a homogenous population of mesenchymal stem cells that have been isolated, purified, and then culturally expanded. The specification of the application which is used construe this language teaches at p. 5, ll. 1-12 that: [m]esenchymal stem cells (MSCs) can be derived from marrow, periosteum, dermis and other tissues of mesodermal origin. The mesenchymal stem cells can be isolated and prepared according to methods known in the art, for example, a process for isolating, purifying, and expanding the marrow-derived mesenchymal stem cells in culture, i.e. in vitro, is described in U.S. Patent Nos. 5,197,985 and 5,226,914 and PCT Publication No. WO 92/22584 (1992) as well as numerous literature references by Caplan and Haynesworth. The stem cells may be isolated from other cells by density gradient fractionation, such as by Percoll gradient fractionation. The human mesenchymal stem cells also can include a cell surface epitope specifically bound by antibodies from hybridoma cell line SH2, deposited with the ATCC under accession number HB10743l antibodies form hybridoma cell line SH3, deposited with ATCC under accession number HB10744; or antibodies from hybridoma cell line SH4, deposited with the ATCC under accession number HB10745 (Emphasis added).

As discussed above, Applicants note that the mesenchymal stem cells taught in the present application are a specific cell type, possessing defined characteristics recognized by those skilled in the art, as evidenced by Majumdar *et al.* Majumdar *et al.* discuss the differences between isolated, purified mesenchymal stem cells

(MSCs) and marrow-derived stromal cells (MDSCs) (see Abstract). Majumdar *et al.* teach that MSCs are morphologically distinct from MDSC cultures, and flow cytometric analyses show that MSCs are a homogeneous cell population (*Id.*; Figs. 1 & 2). In addition, MSCs were found to have greater steady state levels of cytokine expression than MDSCs (*See* Abstract and Fig. 3). Majumdar *et al.* disclose a method for isolating, purifying and culturally expanding MSCs that utilizes Percol density centrifugation as a purification step (p58, first full para.). The use of Percol purification is discussed in the present specification at p. 5, ll. 6-7 and p. 9, ll. 29-30.

As discussed above, the homogenous population of mesenchymal stem cells of the present application represent a well characterized isolated, purified and culturally expanded cell population which can be prepared in a reproducible manner in contrast to the heterogeneous stromal cell cultures described by Prockop.

Similar to the above rejection based on Nolta *et al*, the Office Action relies on inferences drawn from Prockop and/or Wikipedia to support the contention that the adherent mesodermal cells taught by Wells *et al.* are isolate, purified and expanded mesenchymal stem cells. As discuss above, Prockop does not support the argument that mesenchymal stem cells have been isolated, purified, and expanded. Contrary to the position taken by the Office Action, Prockop teaches that there are advantages to utilizing isolation and purification methods as opposed to the "crude procedure of Freidenstein," i.e. the isolation of marrow stromal cells via adherence to plastic alone that is also used by Wells *et al.* (see p72, col. 1, para. 3 and col. 2, para. 2). These advantages include that "the isolated cells are either clonal or nearly clonal, they express small amounts of bone cell markers such as alkaline

phosphatase, and they can be induced in culture to express large amounts of the same markers…" (p72, col. 2, para 2).

In addition, the Office Action argues that the terms "Mesenchymal stem cell" and "Marrow stromal cell" have been used interchangeably in the art as evidenced by Wikipedia, the free encyclopedia. However, Applicants respectfully submit that the statements found in the cited Wikipedia page are not factually accurate and a person of skill in the relevant art would recognize that the homogenous population of mesenchymal stem cells that have been isolated, purified, and then culturally expanded are distinct from the marrow stromal cells taught by Wells *et al.*

As discussed above, Majumdar *et al.* discuss the differences between isolated, purified mesenchymal stem cells (MSCs) and marrow-derived stromal cells (MDSCs) (see Abstract). Majumdar *et al.* teach that MSCs are morphologically distinct from MDSC cultures, and flow cytometric analyses show that MSCs are a homogeneous cell population (*Id.*; Figs. 1 & 2). In addition, MSCs were found to have greater steady state levels of cytokine expression than MDSCs (*See* Abstract and Fig. 3). Majumdar *et al.* disclose a method for isolating, purifying and culturally expanding MSCs that utilizes Percol density centrifugation as a purification step (p58, first full para.). The use of Percol purification is discussed in the present specification at p. 5, ll. 6-7 and p. 9, ll. 29-30.

Applicants note that the present application differentiates between MSCs and Dexter stroma. Applicants further note that Dexter stroma as discussed in the present specification is the same as the MDSCs discussed by Majumdar *et al.* (See Majumdar *et al.* p58, para. spanning col. 1-2 and Experimental Example p10, ll.

13-23.). Thus, one skilled in the art would recognize that MSCs are a distinct cell population from the heterogeneous stromal cells discussed by Wells *et al.*

Therefore, claim 5 of the present invention is not anticipated by Wells *et al.* because like Nolta *et al.*, Wells *et al.* do not teach the co-culturing human hematopoietic progenitor cells with a homogenous population of human mesenchymal stem cells isolated, purified and then culturally expanded from human mesoderm tissue…" At best Wells *et al.* teach the culturing of CD34 cells in the presence of an irradiated mesodermic tissue.

Accordingly, Applicants respectfully request that the 35 U.S.C. §102(b) rejection of claim 5 be withdrawn because Wells *et al.* as evidenced by Prockop do not teach all the limitations of claim 5. Claims 2 and 4 depend either directly from claim 5, and therefore should be allowed because of the aforementioned deficiencies in the rejection with respect to claim 5 and because of the specific limitation recited in claims 2 and 4.

In addition, claim 6 has been canceled, thereby rendering the §102(b) rejection of claim 6 moot.

In view of the foregoing, it is respectfully submitted that the present application is in a condition of allowance and allowance of the present application is respectfully requested.

Please charge any deficiency or credit any overpayment in the fees for this matter to our Deposit Account No. 20-0090.

Respectfully submitted,

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